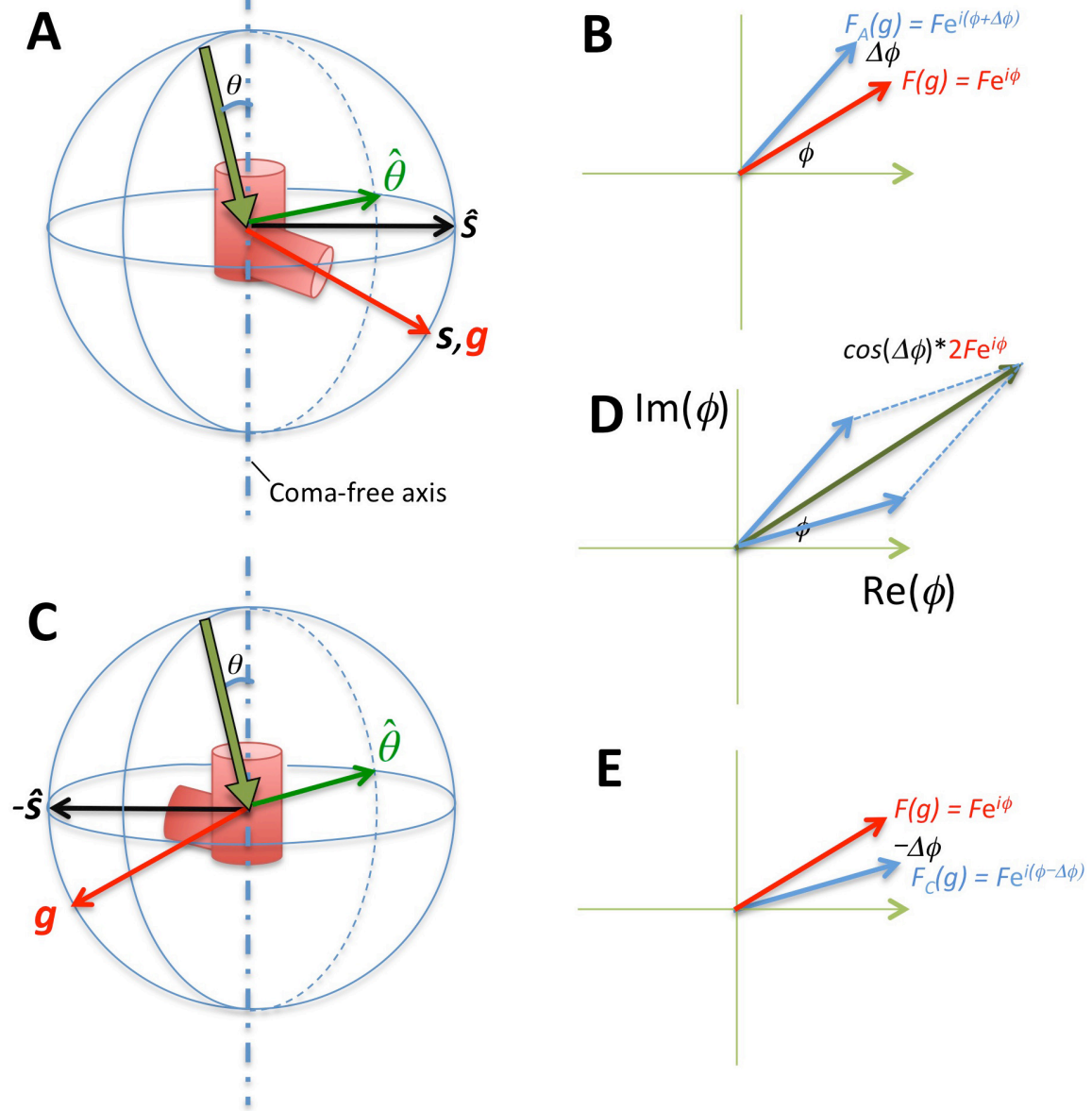Figure 1S Diagrams illustrate how the sum of structure factor $F(g)$ gives the correct phase from two particles of specific orientation relationship. (**A**) The unscattered beam shown as a thick light green arrow is tilted from the coma-free axis by θ, and the beam tilt direction unit vector $\hat{\theta}$ (light green arrow) is shown on the equatorial plane of the unit vector sphere. The red solid object represents a particle that we image. Its structure factor F is a function of the structure spatial frequency vector g that rotates as the particle rotates. For a spectral frequency vector based on the optical space s, its projection on to the equator at unit length defines $\hat{s}$. Together, $\hat{\theta} \cdot \hat{s}$ defines the extent to which coma affects the structure factor found at s. (**B**) shows an Argand diagram of the distorted Fourier transform at g, $F_A(g)$, that is derived from the image taken with the beam tilt. It has a phase shift $\Delta\phi$ from $F(g)$ as described in Equation 1. When the particle is rotated by

180 ° against the coma-free axis, **g** is in the direction defined by $-\hat{s}$. Therefore, the dot product becomes $-\hat{\theta}\bullet\hat{s}$. (**E**) shows Argand diagram of this case. As a result, the two distorted structure factors sum up to no phase distortion in (**D**)

Table S1. Experimental design

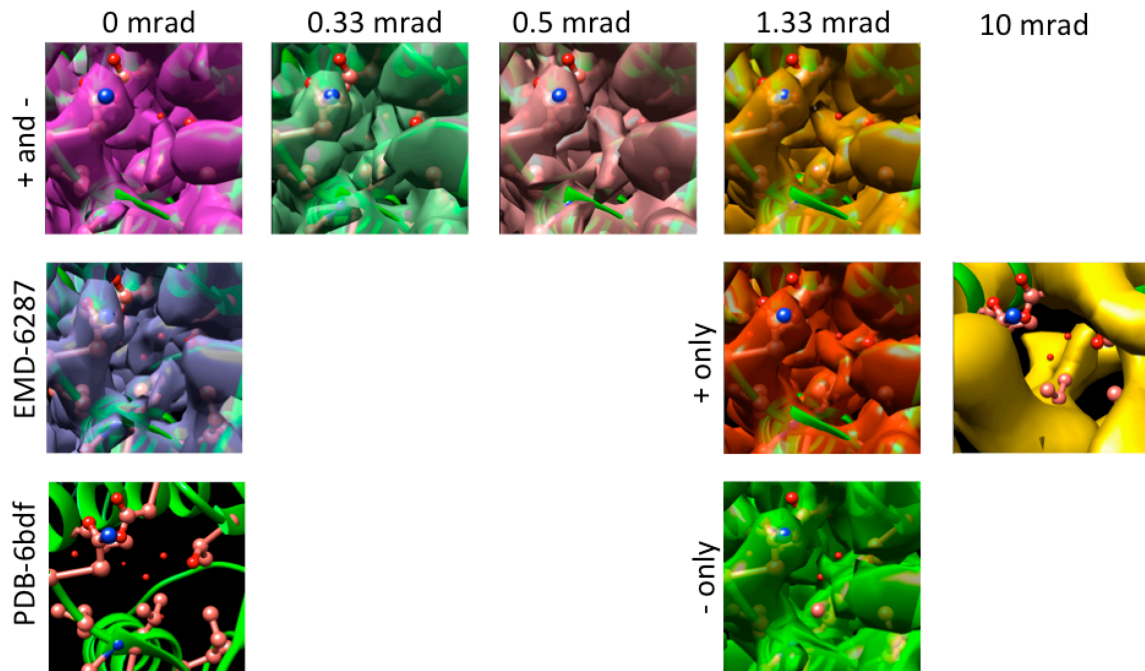| Session	Targeting scheme	Maximal Beam Tilt (mrad)	Beam Tilt measurement	Exposure time (s)	Frame time (s)
Expt 1	Figure 2A	0.33	(Cheng, 2016)	5 or 7	0.2
Expt 2	Figure 2B	1.3	Diffraction ring	6	0.1
Expt 3	Figure 2A	5	N/A	6	0.2
Expt 4	Figure 2A	10	N/A	6	0.2

Table S2. Statistics for various ~1.0 Å/pixel datasets

Session	Beam Tilt (mrad)	Beam-Image Shift Magnitude (μm)	# of particles in the final 3D class	FSC0.143 @single tilt (Å)	B-factor (Å²)	ResLog slope (1/Å)	FSC0.143@tilt-combined (Å)	FSC0.143@0tilt from the same grid (Å)
emdb-6287	0	0		N/A			N/A	2.8
Expt 1	0	0	32k	2.89	N/A		N/A	2.89
Expt 1	0.33	+/- 1.7	48k	N/A	N/A		2.89	2.89
Best	0.33	+/- 1.7	490k	N/A	147		2.64	N/A
Expt 2	0	0	114k	2.74	164	0.0287	N/A	2.74
Expt 2	0.5-&	-2.6	104k	2.80	165	0.0266	2.92•	2.74
Expt 2	0.5+&	+2.6	111k	3.05	191	0.0239		2.74
Expt 2	1.3-&	-2.6	107k	3.09	192	0.0190	3.16•	2.74
Expt 2	1.3+&	+2.6	105k	3.39	228	0.0119		2.74
Expt 3	5	+/- 1.7	243k	4.07	365		4.07	N/A
Expt 4	10	+/- 1.7	72k	5.37	712		5.37	N/A

- Number of particles doubled by combining +/- tilts
- Change of row shading indicates change to a new grid.

& The off-axis beam tilt cannot be estimated in Experiment 2 from beam displacement in the objective lens focal plane nor can it be obtained from a Zemlin tableau due to the large misalignment. Based on the resulting resolution, we predict an underestimate of the beam tilt in one direction (noted by -) and an over-estimate of the same in the other direction (+).

Another example of water peaks

	0 mrad	0.33 mrad	0.5 mrad	1.33 mrad	10 mrad

+ and -

EMD-6287

+ only

PDB-6bdf

- only

307N.water and 302N.water